Journal of Investigational Biochemistry
www.scopemed.org
DOI: 10.5455/jib.20170120093510

Original Research

# Unripe *Carica papaya* and its effect on some antioxidant enzymes and factors associated with vascular disease in models

**Idiakheua O. Dennis, Iyawe O. Hanson**

Department of Biochemistry, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, P.M.B. 14 Esan West LGA, Edo State, Nigeria

Address for correspondence: Iyawe O. Hanson, Department of Biochemistry, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, P.M.B. 14 Esan West LGA, Edo State, Nigeria. E-mail: iyawehanson@yahoo.com

**Received:** August 16, 2016
**Accepted:** December 16, 2016
**Published:** February 28, 2017

## ABSTRACT

**Background:** *Carica papaya* is a member of the Caricaceae family. The ripe fruits are juicy and rich in nutrients. The unripe fruits are seldom consumed. There are several unverified claims of traditional usage of unripe *C. papaya* in phytotherapy. Ripe papaya is used to improve digestive and abdominal disorders, treat dyspepsia, hyperacidity, dysentery, and constipation. Extracts of unripe *C. papaya* contain considerable phytochemicals and it is applied to treat diuresis or can be used as a mild laxative and to stimulate lactation. Information on the consumption of raw unripe papaya is observed to be scarce. **Objectives:** The thrust of this study was to examine the possible outcome of consuming mature and unripe *C. papaya* on some antioxidant enzymes and lipid profiles in rats. To conduct this study, a 4 × 6 experiment was designed, comprising four groups of six rats per group. Control (Group A) was fed with standard feed. Group B received standard feed with unpeeled blend of *C. papaya*. Group C received standard feed with peeled blend of *C. papaya*, while Group D was given standard feed with boiled and unpeeled blend of *C. papaya*. The feed–papaya mix ratio was 80:20 in all cases. Feeding took 4-week duration, after which blood samples were harvested and processed for analysis. **Materials and Methods:** Nutrient composition of feed blends was done with Association of Official's Analytical Chemists methods. Serum total cholesterol (TC), triglyceride, and high-density lipoprotein (HDL) were determined with standard assay kits. Serum catalase activity, superoxide dismutase (SOD) activity, glutathione reductase (GR), and glutathione peroxidase (GPx) were assayed using standard procedures. **Results:** Weight gains were observed among all groups. There was significant ($P < 0.05$) TC reduction among test animals compared to control. Both HDL and low-density lipoprotein were reduced ($P < 0.05$) in comparison to their controls. There were significant ($P < 0.05$) variations among the antioxidant enzymes under the study. **Conclusion:** The import of these data is to the effect that matured and unripe *C. papaya* has the potential to reduce some risk factors in vascular disease in rats and challenge antioxidant enzymes according to their respective processing method.

KEY WORDS: Antioxidants, cholesterol, pawpaw, vascular disease

## INTRODUCTION

Endothelial cells of the inner lining of a blood vessel provide an anticoagulant barrier between the vessel wall and blood. It also serves as a selective permeability barrier. It is a unique multifunctional cell with critical basal and inducible metabolic and synthetic functions. The cell reacts with physical and chemical stimuli within the circulation and regulates hemostasis, vasomotor tone, as well as immune and inflammatory responses. Endothelial cell injury or dysfunction is a hallmark of many pathologic states [1].

Mammalian cells produce energy by reducing molecular oxygen. During this process, reactive intermediates such as superoxide anion, hydroxyl radicals, and hydrogen peroxide are generated. Due to their highly biologically reactive properties, these molecules may interact with proteins, lipids, and DNA. Their excessive production has been implicated in the pathogenesis of various diseases. In addition, there are reports that vascular production of superoxide is increased in hypercholesterolemia, diabetes mellitus, hypertension, and cigarette use [2,3].

The vascular system is a network of blood vessels which includes the arteries, veins, and capillaries that carry blood to and from the heart. Challenges of the vascular system are legion, ranging from atherosclerosis, blood clots, and weakened vessels, occasioned by fatty deposits. High cholesterol level has been linked to peripheral vascular disease (PVD), which refers to diseases of blood vessels outside the heart and brain. In PVD, fatty deposits build up along artery walls and affect

blood circulation to the legs and feet, leading to stroke. Oxidative stress leads to oxidation of low-density lipoprotein (ox-LDL), in which uptake by macrophages is easier compared to reduced lipoproteins (red-LDL). The main sources of oxidative substances and reactive oxygen species (ROS) in atherosclerotic vessels are macrophages and smooth muscle cells. Indeed, hypercholesterolemia stimulates the production of superoxide anion radicals ($O_2^-$) from the smooth muscle cells or vessels; this event leads to increased ox-LDL with expression and function of oxidant and antioxidant enzymes. These enzymes may be nicotinamide adenine dinucleotide phosphate oxidase, endothelial nitric oxide synthase, xanthine oxidase, myeloperoxidase, superoxide dismutase (SOD), catalase (CAT), thioredoxin reductase, and glutathione peroxidase (GPx). Studies have underlined the importance of dysregulated oxidant and antioxidant enzymes for the development and progression of atherosclerotic disease in animals and humans. It has been suggested that specific pharmacological modulation of key enzymes involved in the propagation of oxidative stress rather than using direct antioxidants may be an approach to reduce oxygen radical load in the vasculature and subsequent disease progression [4], although another research finding had shown that PVD present alone does not alter key antioxidant enzyme activity [5].

Fruits and vegetables are generally low in cholesterol and are recommended as part of a healthy diet. These items may reduce chronic vascular diseases particularly, by means of their protective constituents such as potassium, folate, vitamins, fiber, and other phenolic compounds. These nutrients act through a variety of mechanisms, such as reducing oxidative stress, improving lipoprotein profile, lowering blood pressure, increasing insulin sensitivity, and improving hemostasis regulation [6-8].

Cholesterol undergoes free radical-mediated oxidation through hydroperoxide formation to oxysterols, which are suspected of being initiators of atherosclerotic plaques. Oxysterols inhibit hydroxy methylglutaryl-CoA reductase activity resulting in a decreased cholesterol concentration in the cell membrane, which leads to endothelial membrane injury and possible premature cell death [9].

One popular fruit in the tropic and subtropical regions is *Carica papaya*. The nutritional values of *C. papaya* have been documented and the extracts of unripe fruit have been reported to contain terpenoids, alkaloids, flavonoids, carbohydrates, glycosides, saponins, and steroids. These nutrients improve cardiovascular system, protect against heart diseases, heart attacks, strokes, and prevent colon cancer [10].

Other studies conducted on *C. papaya* are on its antibacterial activity [11], traditional and medicinal uses [10]. Evaluation of *C. papaya* leaves had been described [12]. Antioxidant and immunostimulant effects had also been documented [13]. Anti-hyperlipidemic activity of *C. papaya* seed had been given [14]. Platelet augmentation activity of leaves had been described [15]. Toxic effect of the plant bark has been described [16]. Toxicity studies of unripe *C. papaya* had been conducted [17], and the effect of aqueous extract of *C. papaya* leaves on liver and blood cells has been proposed [18]. *C. papaya* is reported to be one of the most effective sources of natural medicine and widely used in pharmacological applications [19]. These researchers [11-18] concentrated their efforts on the plant leaves, seeds, and roots, without adequate consideration on the consumption of unripe fruit of *C. papaya*, therefore leaving a gap specifically on the influence of wholesome consumption of unripe *C. papaya* on vascular dysfunction. This gap is what this paper seeks to address, with a view to provide empirical data for reference purposes.

## MATERIALS AND METHODS

### Plant Fruit Authentication and Processing

Matured fresh unripe *C. papaya* fruits were obtained from a local papaya plantation in the University town and were identified and authenticated by a qualified taxonomist in Ambrose Alli University for future reference. The fruits were washed and divided into three parts. Part A was peeled, chopped in bits, and oven dried (Group B). Part B was unpeeled, chopped in bits, and oven dried (Group C). Part C was peeled, chopped in bits, boiled for 30 min, and oven dried (Group D). In all cases, the cream-colored seeds inside the fruits were discarded. Oven drying was done at a minimal temperature of 40°C until constant weights were obtained. Afterward, all samples were pulverized and used, respectively, for feed blending. Purchased commercial feed was used as reference (Group A).

### Animals

Male Wistar albino rats (195-225 g) obtained from the animal house of College of Medicine, Ambrose Alli University, Ekpoma, were used for the study. They were kept in cages in well-ventilated house, temperature of 27-30°C, 12 h natural light, and 12 h darkness, with free access to tap water and dry rat commercial hybrid feeds (standard diet). They were allowed to acclimatize for a week prior to the experiment during this period and they were observed for signs of weakness and poor feeding.

### Experimentation

After acclimatization period, the animals were allocated to four groups (A-D) of five rats each according to weight proximity. Control group received standard commercial diet. The remaining groups were given blended rat feed for 28 days. Feed blends comprising standard diet and processed *C. papaya* meal were in 80:20 ratio. The treatment of experimental animals was in accordance with the National Institute of Health guidelines for the Care and Use of Laboratory Animals [20]. At the end of 28-day feeding period, the rats were weighed again and sacrificed by making incisions at their cervical regions with sterile blade after being to sleep using ether in a closed container. Their organs were excised and their weights were measured. Blood was harvested by cardiac puncture into anticoagulant-free tubes with corks for biochemical parameters.

### Biochemical Analysis

*Proximate analysis*

Experimental feed blends were analyzed for moisture, protein, fat, ash, fiber, and nitrogen-free extract by the methods of Association of Official's Analytical Chemists [21].

*Cholesterol assays*

Commercial reagent kits for the assay of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDLc) were as supplied by Randox Diagnostics, UK.

*Enzyme activity assays*

CAT was estimated by the method described by Singha [22], and a unit of CAT is defined as the amount that will decompose 1.0 $\mu$mole of $H_2O_2$ per minute at specified conditions. SOD activity was determined as described by Misra and Fridovich [23], and one unit of SOD activity is defined as that amount of enzyme required to exhibit 50% dismutation of the superoxide radical. Glutathione reductase (GR) activity was measured using the method of Goldberg *et al.* [24]. GPx activity was measured using an adaptation of the spectrophotometric method [25].

### Statistical Analysis

Values are presented as mean ± standard error of mean. One-way ANOVA followed by Tukey–Kramer posttest was applied for data evaluation, and the level of significance was set at $P \leq 0.05$.

## RESULTS

Proximate analysis of the prepared meals of *C. papaya* and the average weight gains of animals are shown in Tables 1 and 2, respectively. There were significantly ($P < 0.05$) low carbohydrates and proteins in experimental diets relative to control group. Significant ($P < 0.05$) high crude fiber, ash, and moisture were observed with higher lipid contents in unpeeled and oven-dried papaya-mixed feed. The observable average weight gain per group ranges from 5.30 ± 2.91 g in Group D to 22.00 ± 6.80 g in control group. Highest weight gain among the experimented group was recorded in Group B with 13.40 ± 3.08 g. Decreases ($P < 0.05$) were recorded for cholesterol, TGs, HDL and LDL among the treated groups in comparison with control, as indicated in Table 3. Group D recorded the least cholesterol, TGs, and LDL values as well as highest HDL as compared to other treated groups, except control.

The results of antioxidant enzymes assessed under the study conditions are shown in Table 4. Group C recorded the highest SOD activity followed by Group D as against control. CAT and GPx activities among all the treated groups were statistically significantly ($P < 0.05$) reduced in comparison with control. However, the activity of GR in test animals' groups was higher ($P < 0.05$) compared to control group.

## DISCUSSION

Proximate analysis conducted on test feed using *C. papaya* showed high protein contents among test groups. However, the protein values were low relative to control. The justification for these observed reductions in protein contents may be attributable to papaya factor. At present, there are conflicts in reports on the protein contents of unripe *C. papaya* fruits, as protein values ranging from 0.4% to 44% for unripe papaya fruit pulp had been reported [26-29]. The low protein content in feed blend is an indication of low protein content in *C. papaya*, as fruits are generally not good sources of proteins except for vitamins and minerals [26,30].

The differences in carbohydrate levels in formulated feed could be linked to reduction in quantities used in feed mix of test groups. This is again made manifest in the crude fiber contents of experimental feed as there are about 5-fold increase in crude fiber contents of test feeds as against the 4:1 feed mix ratios. Despite the reduction in carbohydrate levels, the values are high enough to affirm unripe *C. papaya* as carbohydrate-rich fruit as reported earlier [28].

The ash content which gives indication of the mineral composition in food materials was high in formulated feeds, as compared with control due to *C. papaya* inclusions, as the fruits are minerals and vitamin rich [30].

The significant high fat contents in Groups B and C feeds are possibly due to concentrations arising from drying while lipids of Group D may have been lost due to steaming. The low fat contents as against recommended 10% in formulated feeds are the assurances of reduced susceptibility to oxidative rancidity [31]. In addition, the high lipid contents in Groups B and C feed blends may also increase the palatability of food by absorbing and retaining flavors [30,32].

High intake of energy-dense foods promotes weight gain. These foods are not only highly processed but they are also low in fiber, high in fat, and sugars. Epidemiological evidence suggests that nutritional lifestyle may program the risk of chronic degenerative diseases such as obesity, increased blood pressure, endothelial dysfunction, glucose intolerance, and renal impairment [33]. The progressive reductions in body weights [Table 2] observed in animal feeds with test meal as against control indicated the potentials of the formulated diet to regulate excessive weight

**Table 1: Proximate analysis (%) of the different *Carica papaya* meals**

| Parameters | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| Carbohydrate | 70.58±3.22$^{a}$ | 60.70±0.92$^{b}$ | 56.02±0.65$^{c}$ | 62.52±0.19$^{b}$ |
| Protein | 19.50±0.22$^{a}$ | 14.91±0.04$^{b}$ | 14.77±0.03$^{b}$ | 15.01±0.02$^{b}$ |
| Fats | 3.80±0.12$^{a}$ | 4.80±0.03$^{a,b}$ | 5.31±0.02$^{b}$ | 3.93±0.03$^{a}$ |
| Fiber | 3.00±0.10$^{a}$ | 15.77±0.03$^{b}$ | 15.67±0.04$^{b}$ | 14.61±0.02$^{b}$ |
| Moisture | 3.40±0.20$^{a}$ | 6.61±0.85$^{b}$ | 4.48±0.61$^{b}$ | 5.15±0.27$^{b}$ |
| Ash | 2.75±0.01$^{a}$ | 3.53±0.02$^{b}$ | 3.75±0.02$^{b}$ | 3.93±0.03$^{b}$ |
| Dry matter | 96.60±0.25$^{a}$ | 93.39±4.85$^{a}$ | 95.52±7.82$^{a}$ | 94.85±5.85$^{a}$ |

Results on the same row with different letters are significantly different

Table 2: Weight (g) of the rats before and after feeding with *Carica papaya* diet

| Weight of animals | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| Before feeding | 86.90±3.22 | 62.30±2.50 | 72.10±1.18 | 76.30±2.80 |
| After feeding (g) | 136.81±11.30 | 87.40±5.20 | 95.39±3.95 | 96.42±3.80 |
| Weight gain per group | 22.00±6.80[a] | 13.40±3.08[b] | 9.08±2.48[c] | 5.30±2.91[d] |

Results on the same row with different letters are significantly different

Table 3: Lipid profile (mg/dL) of rats fed with blends of *Carica papaya* diet

| Parameters | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| Cholesterol | 55.62±0.28[a] | 44.75±0.21[b] | 27.32±0.21[c] | 10.54±0.13[d] |
| Triglycerides | 47.08±1.09[c] | 10.23±4.59[a] | 9.73±2.91[a] | 5.94±1.33[d] |
| HDL | 11.29±0.11[a] | 11.91±0.16[c] | 11.48±0.11[d] | 12.40±0.11[b] |
| LDL | 34.19±0.53[a] | 21.41±1.55[b] | 6.89±0.54[c] | 2.95±0.32[d] |

Results on the same row with different letters are significantly different. HDL: High-density lipoprotein, LDL: Low-density lipoprotein

Table 4: Some antioxidant enzyme activity (units/mL) in rats fed with *Carica papaya* diet

| Parameters | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| SOD | 7.05±0.27[b] | 4.31±0.02[d] | 13.87±0.09[a] | 6.37±0.13[b] |
| CAT | 51.67±0.23[a] | 10.58±0.03[d] | 17.48±0.05[c] | 18.37±0.06[c] |
| GR | 29.04±2.01[c] | 6.83±0.45[d] | 35.31±0.27[b] | 41.16±0.44[a] |
| GPx | 6.51±0.50[a] | 5.48±0.30[b] | 4.11±0.10[c] | 3.48±0.20[d] |

Results on the same row with different letters are significantly different. SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, GR: Glutathione reductase

gain in adult rats. The most adducible reason for weight gain reductions may be possible variations in starch and fiber contents which may have arisen from differentials in processing methods of *C. papaya* included in feed.

Dyslipidemias characterized by disorders in the levels of circulating TC, LDL-cholesterol (LDLc), diminished fraction of HDLc, and TG have been implicated in cardiovascular and vascular diseases [34].

The significant ($P < 0.05$) reductions in TC, TG, and LDL and the significant ($P < 0.05$) increase in HDL are in consonant with earlier study [14]. The variants in the studies used for comparison are seeds and whole fruits with seeds excluded. The observed hypolipidemic effect of *C. papaya* may be due to phytochemical contents of the fruits. Diets high in fruits, vegetables, whole grains, and legumes are rich in bioactive compounds which are associated with reducing the risk of vascular diseases [35]. In particular, high levels of TG and LDLs in Group A, which can be associated with coronary artery disease [14,36], are ameliorated with formulated feed containing *C. papaya*.

Among the test groups, Group D had the lowest result for vascular disease indicators including the highest for HDLc. Rationalization for this may be articulated on grounds of processing technique which incorporated 30 min boiling. It will appear that boiling mature and unripe *C. papaya* may impact latent attributes which perhaps become dominant against disposition to vascular diseases.

Low levels of ROS are indispensable in normal body processes. Higher amounts, however, may play a role in a number of human diseases. As safeguard against the accumulation of ROS, several antioxidant enzymes such as GPx, GR, CAT, and SOD exist. In addition, nutrient-derived antioxidants such as ascorbic acid, tocopherols, carotenoids, glutathione, and lipoic acid also exist [37]. Earlier studies report antinutrient contents of some fruits including *C. papaya* to be within tolerable levels [29,38]. The significantly increased SOD activity in Group C animals and the significant reductions in Groups B and C animals against control may be due to heat stress. Heat stress had been reported to be of concern for animal production as well as human life, especially in hotter regions of the world, as well as environmental factors that could be responsible for stimulating ROS production [39]. However, the relationship between heat stress in processed plant fruits and superoxide radical production following its consumption by animals remains to be explained.

GR plays an important role in protecting biological cell membranes against oxidative damage by increasing the level of reduced glutathione (GSH). The hereditary deficiency of the enzyme is extremely rare [40]. The increases ($P < 0.05$) observed in Groups C and D against controls are probably indicative of the need for cells to produce GSH to meliorate the concomitant increases in SOD activity and CAT activity, except in the case of Group D where SOD activity did not differ ($P < 0.05$) from control. It is likely that individual enzyme activity may have culminated in the significant stability of oxidative stress as observed in the low values of oxidized glutathione. All put together, this finding is suggestive of the potentials of *C. papaya* as used in this investigation to prevent vascular malady.

## REFERENCES


1. Sumpio BE, Riley JT, Dardik A. Cells in focus: Endothelial cell. Int J Biochem Cell Biol 2002;34:1508-12.
2. Nedeljkovic ZS, Gokce N, Loscalzo J. Mechanisms of oxidative stress and vascular dysfunction. Postgrad Med J 2003;79:195-9; 198-200.
3. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47-95.
4. Wassmann S, Wassmann K, Nickenig G. Modulation of oxidant and antioxidant enzyme expression and function in vascular cells. Hypertension 2004;44:381-6.
5. Jandric-Balen M, Bozikov V, Bistrovic D, Jandric I, Bozikov J, Romic Z, *et al.* Antioxidant enzymes activity in patients with peripheral vascular disease, with and without presence of diabetes mellitus. Coll Antropol 2003;27:735-43.
6. Van Duyn MA, Pivonka E. Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: Selected literature. J Am Diet Assoc 2000;100:1511-21.
7. Bazzano LA, Serdula MK, Liu S. Dietary intake of fruits and vegetables and risk of cardiovascular disease. Curr Atheroscler Rep 2003;5:492-9.
8. Pereira MA, O'Reilly E, Augustsson K, Fraser GE, Goldbourt U, Heitmann BL, *et al.* Dietary fiber and risk of coronary heart disease:

A pooled analysis of cohort studies. Arch Intern Med 2004;164:370-6.
9. Hubbard RW, Ono Y, Sanchez A. Atherogenic effect of oxidized products of cholesterol. Prog Food Nutr Sci 1989;13:17-44.
10. Aravind G, Debjit B, Duraivel S, Harish G. Traditional and medicinal uses of *Carica papaya*. J Med Plants Stud 2013;1:7-15.
11. Doughari JH, Elmahmood AM, Mazara S. Studies on the antibacterial activities of root extract of *Carica papaya* L. Afr J Micbiol 2007;1:37-41.
12. Ayoola PB, Adeyeye A. Phytochemical and nutrient evaluation of *Carica papaya* (pawpaw) leaves. IJRRAS 2010;5:325-8.
13. Kadry MS. Antioxidant and immunostimulant effect of *Carica papaya* Linn. Aqueous extract in acrylamide intoxicated rats. Acta Inform Med 2012;20:180-5.
14. Nwangwa EK, Ekhoye EI. Anti-hyperlipidemic activity of aqueous extract of *Carica papaya* seed in albino rats fed with high fat diet. Curr Trends Technol Sci 2013;2:262-6.
15. Swati P, Supritha S, Rama B, Shridhar N. Evaluation of platelet augmentation activity of *Carica papaya* leaf aqueous extract in rats. J Pharm Phytochem 2013;1:57-60.
16. Duru MK, Amadi BA, Amadi CT, Lele KC, Anudike JC, Chima-Ezika OR, *et al.* Toxic effect of *Carica papaya* bark on body weight, haematology, and some biochemical parameters. Biokemistri 2012;24:67-71.
17. Oduola T, Adeniyi FA, Ogunyemi EO, Bello IS, Idowu TO, Subair HG. Toxicity studies on an unripe Carica papaya aqueous extract: Biochemical and haematological effects in wistar albino rats. J Med Plant Res 2007;1:001-4.
18. Nwiloh BI, Nwinuka NM, Monanu MO. The effect of aqueous extract of *Carica papaya* leaves on liver enzymes and blood cell counts of normal albino rats. Int J Biol Chem Sci 2009;3:561-6.
19. Adam A, Elgadir MA, Salama M. *Carica papaya* as a source of natural medicine and its utilization in selected pharmaceutical applications. Int J Pharm Pharm Sci 2014;6:880-4.
20. National Institute Health. National Research Council Guide for the Care and Use of Laboratory Animals. Publication. No. 85-123 (Rev.). Bethesda, MD: National Institute Health; 1985.
21. AOAC. Official Methods of Analysis of the Association of Official's Analytical Chemists. 17th ed. Arlington, Virginia: Association of Official Analytical Chemists; 2003.
22. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389-94.
23. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247:3170-5.
24. Goldberg DM, Spooner RJ. Glutathione reductase. In: Bergmeyer HU, editor. Methods in Enzymatic Analysis. Vol. 7. Basel: Verlag Chemie; 1985. p. 258-65.
25. Flohe L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymol 1984;105:114-21.
26. Oloyede OI. Chemical profile of unripe pulp of *Carica papaya*. Pak J Nutr 2005;4:379-81.
27. Karuna SV, Vijaya SK. Nutritive assessment of different plant parts of *Carica papaya* Linn of Jabalpur region. J Nat Prod Plant Resour 2014;4:52-6.
28. Chukwuka KS, Iwuagwu M, Uka UN. Evaluation of nutritional components of *Carica papaya* L. at different stages of ripening. J Pharm Biol Sci 2013;6:13-6.
29. Nwofia GE, Ojimelukwe P, Eji C. Chemical composition of leaves, fruit pulp and seeds in some *Carica papaya* (L.) morphotypes. Int J Med Arom Plants 2012;2:200-6.
30. Odom TC, Udensi EA, Iwe MO. Nutritional evaluation of unripe *Carica papaya* unripe Musa paradisiacal and *Mucuna cochinchinensis,* weaning food formulation. Eur J Biol Med Sci 2013;1:6-15.
31. Food and Agricultural Organization of the United Nations. Grain Legumes in Africa. 3rd ed. Rome: FAO; 1996. p. 82-3.
32. Antia BS, Akpan EJ, Okon EA, Umoren IU. Nutritive and anti nutritive of evaluation of sweet potatoes leaves. Pak J Nutr 2006;5:166-8.
33. Ware S, Voigt JP, Langley-Evans SC. Body composition and behaviour in adult rats are influenced by maternal diet, maternal age and high-fat feeding. J Nutr Sci 2015;4:e3.
34. Coelho VG, Caetano LF, Júnior RD, Cordeiro JA, Souza DR. Lipid profile and risk factors for cardiovascular diseases in medicine students. Arq Bras Cardiol 2005;85. Available from: http://www.scielo.br/pdf/abc/v85n1/en_a11v85n1.pdf. [Last accessed on 2017 Jan 12].
35. Steinberg FM, Cena ER. Soy may help protect against cardiovascular disease. Calif Agric 2011;65:118-23.
36. Chang JJ, Chen TH, Chan P, Chen YJ, Hsu FL, Lo MY, *et al.* The *in vitro* inhibitory effect of tannin derivatives on 3-hydroxy-3-methylglutaryl-coenzyme a reductase on vero cells. Pharmacology 2001;62:224-8.
37. Matés JM, Pérez-Gómez C, Núñez de Castro I. Antioxidant enzymes and human diseases. Clin Biochem 1999;32:595-603.
38. Onibon VO, Abulude FO, Lawal LO. Nutritional and anti-nutritional composition of some Nigerian fruits. J Food Technol 2007;2:120-2.
39. Ahmad M, Yukio A, Craig HW, Masaaki T. Sequential changes in superoxide production, anion carriers and substrate oxidation in skeletal muscle mitochondria of heat-stressed chickens. FEBS Lett 2007;581:3461-7.
40. Chang JC, van der Hoeven LH, Haddox CH. Glutathione reductase in the red blood cells. Ann Clin Lab Sci 1978;8:23-9.



**Source of Support: Nil, Conflict of Interest: None declared.**